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SOME OBSERVATIONS AND CONSIDERATIONS UPON THE MATURATION PHENOMENA OF THE GERM CELLS.¹

THOS. H. MONTGOMERY, JR.

In a series of studies on the spermatogenesis of the Hemiptera, of *Peripatus*³ and of the Amphibia,⁴ I have endeavored to prove the following points :

1. That the chromosomes retain their individuality from generation to generation to that extent that a chromosome of one generation is not a new formation, however much its chemical substance has become changed by metabolism, but represents at least a part of a particular chromosome of the preceding generation.

2. That the first maturation mitosis in the species studied results in the separation from each other of entire univalent chromosomes, and hence is a transverse or reduction division ; while the second maturation mitosis results in the longitudinal splitting of univalent chromosomes, and therefore is an equational division.

3. That the so-called "reduction in number" of the chromosomes is effected before the maturation divisions, by a pairing (conjugation) in the early growth period (synapsis stage) of the spermatocytes, of univalent chromosomes of like volume, each such composite chromosome then being bivalent with relation to the number in the spermatogonia.

4. That this conjugation of univalent chromosomes in the synapsis stage, is a conjugation of paternal with maternal chromo-

¹ Contributions from the Zoölogical Laboratory of the University of Texas, No. 54.

² "The Spermatogenesis in *Pentatoma* up to the Formation of the Spermatid," *Zoolog. Jahrb.*, 12, 1898; "Chromatin Reduction in the Hemiptera: a Correction," *Zoolog. Anz.*, 22, 1899; "A Study of the Chromosomes of the Germ Cells of Metazoa," *Trans. Amer. Phil. Soc.*, 20, 1901; "Further Studies on the Chromosomes of the Hemiptera heteroptera," *Proc. Acad. Nat. Sci.*, Philadelphia, 1901.

³ "The Spermatogenesis of *Peripatus* (*Peripatopsis*) *balfouri* up to the Formation of the Spermatid," *Zoolog. Jahrb.*, 14, 1900.

⁴ "The Heterotypic Maturation Mitosis in Amphibia and its General Significance," *BIOL. BULL.*, 4, 1903.

somes; and hence that the first maturation mitosis separates paternal from maternal chromosomes.

The third and fourth of these conclusions were new; the second has been a matter of much controversy, while the first has a considerable number of cytologists in its support. If these results can be generally established they will give a coherent basis for understanding the part played by the chromosomes in heredity, taken in conjunction with the important conclusion of Van Beneden¹ that the male pronucleus has a number of chromosomes equal to that in the female pronucleus; and of Henking² and O. Hertwig³ that the maturation phenomena correspond very closely in the ovogenesis and spermatogenesis of the same species.

It is not my purpose here to go over the whole controversy and discuss in full the opposing views, for that has been done in my preceding papers, but rather to draw attention to a few of the more important results, and to add some new observations.

I. THE FIRST MATURATION MITOSIS IN AMPHIBIA.

Janssens and Dumez⁴ have very recently reëxamined the spermatogenesis of Amphibia, particularly with regard to my results on *Plethodon* and *Desmognathus*, and decide that my position is both untenable and unproved: that the heterotypic mitosis is an equational division, and not a separation of entire univalent chromosomes as I had maintained. They state (p. 433): "On est tout étonné de ne trouver dans le texte de Thos. H. Montgomery *aucun argument* pour cette thèse." They do not mention my strongest argument at all, namely that the space enclosed by the heterotypic chromosome is a space separating two whole univalent chromosomes, and not a longitudinal split between two halves of a single chromosome, because this space is largest in the earliest stages, and not, as one would expect if it

¹ "Recherches sur la maturation de l'oeuf et la fécondation," *Arch. Biol.*, 5, 1883.

² "Ueber Spermatogenesis und deren Beziehungen zur Eientwicklung bei *Pyrrhocris apterus* M.," *Zeit. wiss. Zool.*, 51, 1890.

³ "Vergleich der Ei- und Samenbildung bei Nematoden," *Arch. mikr. Anat.* 36, 1890.

⁴ "L'Elément nucléinien pendant les cinèses de maturation des spermatocytes chez *Batrachoseps attenuatus* et *Plethodon cinereus*," *La Cellule*, 20, 1903.

were a longitudinal split, smallest at those periods. In other words, I showed that at the earliest stage when the chromosomes can be distinguished, this space is largest, while the true longitudinal split is found in the axis of each arm of a heterotypic chromosome. They add: "*L'auteur continue et, sans en donner la moindre preuve cette fois, que les deux branches de l'anse s'enroulent l'une autour de l'autre pour constituer les dyades enroulées, qui, d'après tous les auteurs, se trouvent dans les spermatoctes I avant la mise au fuseau des chromosomes.*" It is only necessary to rejoin that the comparison of the chromosomes as shown in my consecutive figures is sufficient proof. But when they say that my figures "*sont fort schématisées,*" I simply answer that is not true, and that all were made with great care with the use of a camera lucida.

Janssens and Dumez do, however, bring up one good criticism. They note quite correctly that in the spermatogonia the chromosomes are of unequal lengths, while the two halves of a bivalent heterotypic chromosome are always of equal length. And they reason that if my view were correct that one of the heterotypic chromosomes is formed by the pairing of two univalent chromosomes, that I would have to demonstrate how two such conjugating chromosomes are always of the same length. For they argue that if the heterotypic chromosome were formed by a longitudinal splitting, such a splitting would fully explain the length equality of the two arms of the chromosome.

This is a good criticism, but I find it answered by a study of the relative volumes of the chromosomes in the equatorial plate stage of the spermatogonia. In every case where the pole view shows all the chromosomes lying in one plane, we can determine by carefully drawing them that there are just 12 pairs of chromosomes present, the two of each pair being of equal volume and frequently of similar form. One will find many cases where the pole view of a chromosome plate does not show this distinctly, but that is only when the chromosomes are irregularly arranged, and when their long axes do not lie in the plane of the equator.

A series of figures demonstrate this. Fig. 1 is the only case where all 24 chromosomes were seen in their entirety on pole

view; in Figs. 2-5 only such chromosomes were drawn as could be sent in their whole length; and in none of the figures were any chromosomes omitted that could be seen in their entirety. Two chromosomes of a corresponding volume are marked with the same letter, one in capitals and other in lower case, as, *e. g.*, *A* and *a*. All these cases were drawn, after numerous preliminary sketches, as accurately as possible with the camera lucida, and then those which seemed to correspond were lettered alike. Thus in Fig. 1, the large chromosomes *A* and *a* are alike, but differ in volume from all others; *B* and *b* are markedly alike, *C* and *c*, and all appear clearly paired. Sometimes, as in the case of *G*, *g*, *H*, *h*, the two of a pair appear dissimilar in the drawings, but this is simply because the curvature of one lies in a different plane from that of the other. Thus in Fig. 3 are shown also all 24 chromosomes, but the two marked *x* and *y* were seen so obliquely that it could not be determined whether they were alike. Fig. 6 shows a lateral view of a spermatogenic spindle, showing also a similar pairing of some of the chromosomes; and Fig. 7 shows the same phenomenon in an oblique pole view of a portion of one plate of daughter chromosomes of an early spermatogenic anaphase.

An examination of these Figs. 1-7, shows that the chromosomes are paired according to their volumes, that the two of a pair are very frequently of the same form; and that in most of the cases the two of a pair lie in the spindle close together. The only interpretation for this last condition is that corresponding chromosomes must have been arranged close together in the continuous chromatin spirem of the prophase, so that in the spirem *A* would be next to *a*, *B* to *b*, and so on. But on a study of nuclei in the late prophase (loose spirem) stage, I was not able to determine this positively, for until the chromosomes have shortened to their definitive forms, they are so irregular and twisted that it is practically impossible to determine their exact lengths on sections; probably crush preparations (such as those employed by Sutton) could be used with advantage here. At least there is no doubt of a continuous linin spirem in the spermatogenic prophase and of the arrangement of the chromosomes in a chain along this thread. And it is probable that

similar chromosomes are contiguous in this spirem, since in the metakinesis like chromosomes usually lie near each other; and, as Fig. 7 shows, they retain their contiguity even in the daughter cells.

Therefore we find, what Janssens and Dumez insisted that I should demonstrate, twelve pairs of chromosomes of the same length in the equatorial plate of the spermatogonia.

Now for the proof that such chromosomes unite into pairs in the spermatocytes, an explanation which, according to the Belgian cytologists, "est absolument fantastique et demanderait à être rigoureusement démontrée." On none of my preparations were there stages between the early anaphase of the last spermatogonic mitosis and the synapsis stage of the growth period. In the synapsis stage in *Desmognathus* the long and slender chromosomes are so intricately coiled together that it is impossible in sections to determine their exact relations. The cell has a distinct polarity, the nucleus at one end, at the opposite the greatest mass of cytoplasm containing the idiozome body. When the chromosomes begin to separate sufficiently for their boundaries to be distinguished, each appears as a loop or U with its ends at that part of the nucleus nearest the idiozome body. This is shown in Fig. 8, where only five of the loops are drawn in their entirety. On a transverse section of such a stage (Fig. 9) one finds 24 cross-cut portions of loop; every two of these portions correspond to the two arms of one of the U-shaped loop of Fig. 8. Therefore there are U-shaped chromosomes, with a particular arrangement, to the number of 12; hence they must be bivalent with regard to the 24 chromosomes of the spermatogonia. At this early stage (Fig. 8) there is no sign that the space enclosed by such a loop has arisen by a longitudinal splitting of a chromosome, for in fact the characteristic shape of the loops is the same now as at later stages (Figs. 10, 11). Now this was the main basis of my argument before — an argument that Janssens and Dumez ignore: that if this were a longitudinal split, we should find its commencement in such an early stage.

The true longitudinal split commences in the stage of Fig. 8, is very prominent in that of Figs. 9, 10; this is a clear splitting of each chromatin granule of the arms of each bivalent

chromosome, but the width of this split never becomes wider than that shown in Fig. 11. Janssens and Dumez, as all the workers on amphibian spermatogenesis before them, have entirely overlooked this split of each arm of a bivalent chromosome. With material stained in iron hæmatoxyline, and sufficiently destained, this split, though narrow, is perfectly distinct.

There is a point brought up by Janssens and Dumez to which attention must be drawn. They maintain (1) that there is no regular occurrence of a band of linin (that marked *k* in the figures of my previous paper), joining the two univalent arms composing a U, and placed at the bend of the U; and (2) that the linin spirem appears continuous and not broken into as many segments as there are U-shaped loops. A reëxamination shows me that they are right in regard to there being here a continuous linin spirem. And this is exactly what I gave especial study to proving to be the case in the corresponding stages of *Peripatus*. But I must maintain against these writers, that in *Plethodon* and *Desmognathus*, as in *Peripatus*, there is at no stage in the spermatocytes a continuous chromatin spirem. Sometimes one arm of a U-shaped chromosome seems to be continuous with the end of an arm of another, as in Fig. 11 (and Fig. 5 of my preceding paper). But this is unusual, and generally one finds, as in Figs. 8, 10 and 11, that the ends of the U-shaped chromatin loops are connected with the ends of other loops only by linin. Hence the boundaries of the bivalent chromosomes are perfectly distinguishable in most cases. As to the first point, I admit that the U-shaped loop, which I regard as composed of two univalent chromosomes attached at the bend of the U, does not show in all cases a band of linin at its bend; but it does nevertheless in many cases. However, on this point I did not place great insistence, as Janssens and Dumez maintain.

The U-shaped bivalent chromosomes of Fig. 11 shorten and condense into forms such as shown in Fig. 12. By the condensation of the chromatin the longitudinal split becomes hidden. This is not a remarkable phenomenon, as maintained by the Belgian writers; I and others have described it for frequent cases in arthropods. It reappears in the anaphase of the first maturation mitosis as a split along which the chromosomes

divide in the second mitosis. Finally the definitive shape is reached, as shown in Figs. 13 and 14. One peculiarity has often been described in heterotypic chromosomes: as they are placed in the spindle (Fig. 13), very frequently at the middle of each is one thickening, and this is more frequent than two thickenings. By comparison of a chromosome, such as that of Fig. 14, with earlier conditions, such as those of Fig. 12, it becomes evident that such a thickening corresponds to the separated ends of the U-shaped loop, which have finally come into close juxtaposition.

When we consider these points, we find two important facts: (1) that the twenty-four chromosomes are regularly paired in the spermatogonia, and that there the two of a pair lie close together; and (2) that there is no evidence that the space enclosed by any one of the twelve heterotypic chromosomes has been formed by a longitudinal splitting. In the spermatocytes there are twelve loops of the shape of a U or V. There is a longitudinal splitting, but along the long axis of each loop. The simple explanation of these facts is that in the spermatocytes, in the synapsis stage, there takes place the close conjugation of every two such chromosomes as were found in the spermatogonia; that two together constitute a U-shaped loop; and that therefore the first maturation division results in separating entire univalent chromosomes.

The difference of opinion between Janssens and Dumez and myself is more one of interpretation than of observation, though they did not notice the pairing of the chromosomes in the spermatogonia, nor yet the true longitudinal split. They frankly admit that by their interpretation the reduction in number of the chromosomes remains a mystery. They give no explanation of why there should be regularly disposed U-shaped loops. In assuming that a heterotypic chromosome has been formed by a longitudinal splitting, instead of by a junction of two univalent chromosomes, they contend for a kind of splitting very wide at the middle of the chromosome, but narrow at its ends; yet no such longitudinal splitting is known in any other case, and its difference is brought out sharply by comparison with the undoubted longitudinal splitting in the chromosomes of the later prophase of the spermatogonia. These heterotypic chromosomes

are different in form from others, just because they represent pairs of univalent chromosomes. If they arose by simple longitudinal splitting, why should they differ so in form from the chromosomes of the spermatogonia, or of the spermatocytes of the second order?

And here my thanks are due to Janssens and Dumez for this critique of my interpretation, because it induced me to study anew the amphibian spermatogenesis, and this reëxamination brought out the fact, strong in support of my position, that the chromosomes are regularly paired in the spermatogonia.

2. THE INDIVIDUALITY OF THE CHROMOSOMES.

By the idea of the maintenance of the chromosomal individuality we do not mean that a chromosome remains chemically unchanged from generation to generation (for after every mitosis a daughter chromosome grows to the size of a mother chromosome before it divides in the next mitosis), but that, despite great metabolic and structural changes, a chromosome of any generation is the descendant of a particular chromosome of the preceding generation, and is not a new formation. A chromosome of one generation represents a chromosome of a preceding, just as much as a cell of one generation represents a particular cell of a preceding generation. This idea was first propounded by Rabl,¹ and has steadily gained in support. The workers on ovogenesis have, for the most part, taken the position that it is not proved; but the reason there is simply the great duration of the growth period of the ovocytes, during a part of which chromosomal boundaries are not distinguishable. The students of spermatogenesis, on the other hand, are fairly unanimous in support of the view.

It is very important that this idea should be firmly established, and certain considerations would show it to be so. There is first the fact that from generation to generation the number of chromosomes remains the same, from the stage of the fertilized egg to that of the ovocyte or spermatocyte of the first order. Even the form of the chromosomes is maintained through these generations, as shown in the case of the cleavage of *Ascaris*. In the sperma-

¹ "Ueber Zelltheilung," *Morphol. Jahrb.*, 5, 1885.

togenesis of some Hemiptera there is no rest stage in the growth period of the spermatocytes, so that the chromosomes can be followed from the spermatogonia to the spermatids. In *Peripatus* this is but a short rest stage, and during it the boundaries of the chromosomes can be readily distinguished.

On the experimental side excellent evidence has been brought in support of this view, particularly by the study of abnormalities, by Boveri,¹ Zur Strassen,² Morgan,³ and Herla.⁴

Evidence fully as strong as that from experimental study has been obtained by observations upon certain modified chromosomes of insects, which are :

3. THE HETEROCHROMOSOMES.

I offer this name to include those peculiarly modified chromosomes to which have been given the names "accessory chromosomes" by McClung,⁵ "small chromosomes" by Paulmier⁶ and "chromatin nucleoli" by myself. They have been described for the Hemiptera by Henking (*l. c.*), Paulmier, and myself; for the Orthoptera by Wilcox,⁷ McClung, Sutton,⁸ de Sinéty;⁹ and for the spider by Miss Wallace.¹⁰

¹ "Zellen-Studien," *Zool. Jahrb.*, 1888; "Befruchtung," *Ergebn. Anat. Entw.*, 1891; "Ueber die Befruchtungs- und Entwicklungsfähigkeit kernloser Seeigel-Eier," *Arch. Entwicklmech.*, 2, 1895; "Mehrpole Mitosen als Mittel zur Analyse des Zellkerns," *Verh. Phys. Ges. Würzburg*, 35, 1902.

² "Ueber die Riesenbildung bei Ascaris-Eiern," *Arch. Entwicklmech.*, 7, 1898.

³ "The Fertilization of Non-nucleated Fragments of Echinoderm Eggs," *Arch. Entwicklmech.*, 2, 1895.

⁴ "Étude des Variations de la Mitose Chez l'Ascaride Mégalocephale," *Arch. Biol.*, 13, 1893.

⁵ "A Peculiar Nuclear Element in the Male Reproductive Cells of Insects," *Zool. Bull.*, 1899; "The Spermatocyte Divisions of the Acrididae," *Bull. Univ. Kansas*, 1900; "Notes on the Accessory Chromosome," *Anat. Anz.*, 20, 1901; "The Accessory Chromosome — Sex Determinant?" *BIOL. BULL.*, 3, 1902; "The Spermatocyte Divisions of the Locustidae," *Kansas Univ. Sci. Bull.*, 1, 1902.

⁶ "The Spermatogenesis of *Anasa tristis*," *Journ. Morph.*, 15, 1899.

⁷ "Spermatogenesis of *Caloptenus femur-rubrum* and *Cicada tibicen*," *Bull. Mus. Comp. Zool. Harvard*, 27, 1895.

⁸ "The Spermatogonial Divisions of *Brachystola magna*," *Kansas Univ. Quarterly*, 9, 1900; "On the Morphology of the Chromosome Group in *Brachystola magna*," *BIOL. BULL.*, 4, 1902.

⁹ "Recherches sur la Biologie et l'Anatomie des Phasmes," *La Cellule*, 19, 1901.

¹⁰ "The Accessory Chromosome in the Spider," *Anat. Anz.*, 18, 1900.

These are chromosomes which preserve to great extent their compact form during the whole growth period of the spermatocytes, and during the rest stages of the spermatogonia, and retain throughout this whole period the deep staining characteristic of the other chromosomes only during the height of mitosis. Thanks to this peculiarity they can be followed with extreme certainty from generation to generation, even during rest stages; and so are splendid evidence for the thesis of the individuality of the chromosomes.

Now there are two kinds of these. In the Orthoptera there is an unpaired one in the spermatogonia, larger than the other chromosomes; in the Hemiptera they are paired in the spermatogonia, and usually smaller than the other chromosomes. Otherwise in their behavior they are very similar in these two groups of insects. To include both these kinds the name "heterochromosomes," as expressing a difference from the other chromosomes, can be advantageously applied; and this would include (1) the "accessory chromosomes" (unpaired in the spermatogonia), and (2) "the chromatin nucleoli" or "small chromosomes" (paired in the spermatogonia). McClung regards them as sex determinants; I have considered them to be chromosomes that are in the process of disappearance, in the evolution of a higher to a lower chromosomal number.

Now these can be followed from generation to generation without in the Hemiptera undergoing those profound changes which characterize the other chromosomes after a mitosis. In the Figs. 15-23 they are the chromosomes marked N , n ; Figs. 16 and 17 show them in the spermatogonic and first spermatocytic mitoses of *Anasa*; Figs. 18, 19 for the same stages in *Corizus*; Figs. 20, 21 for *Trichpepla*; and Figs. 22, 23 for the spermatogonic monaster and late prophase of the first maturation respectively of *Protenor*. In all these cases they can be recognized in mitosis by their much smaller size.

Recently I have found them to occur in the same number and form in the ovogonia; Fig. 15 shows a pole view of the chromosomal plate in the ovogonium of *Anasa*; Fig. 16 a similar view of the spermatogonium of the same species, and the chromosomes marked N and n are found to correspond exactly.

Now for *Protenor* I found that in the spermatogonic chromosomal plate there are always exactly thirteen elements (Fig. 22); the two smallest of these are the heterochromosomes marked N, n ; they are paired, and in the following synapsis stage conjugate to form the smallest bivalent chromosome N, n of Fig. 23; these two chromosomes are then quite similar to the heterochromosomes (chromatin nucleoli) of the other Hemiptera. But there is a large element in the spermatogonium (X , Fig. 22), unpaired there, and which does not conjugate with any other chromosome during the synapsis stage, but remains unpaired in the spermatocyte (X , Fig. 23). This element I called the "chromosome x ." Now, as McClung has also pointed out, this chromosome behaves exactly as does an accessory chromosome in the Orthoptera, being unpaired in the spermatogonia, and not conjugating with any other chromosome during the synapsis stage. Therefore in *Protenor* occur both kinds of heterochromosomes, the small paired ones, N and n , and the large unpaired one, X . N and n are "chromatin nucleoli" according to my terminology, while X is an "accessory chromosome"; thus both kinds of heterochromosomes occur in the same cell, and their likenesses and differences were fully described by me for this species. Both are recognizable through the whole growth period of the spermatocytes by their compact form and deep staining; but only the small pair, N and n , can be recognized in the rest stage of the spermatogonia. In three other Hemiptera, *Alydus*, *Harmostes* and *Edancala* I showed that in the spermatogonia occurred also an uneven number (thirteen) of chromosomes; but in these the odd chromosome does not maintain its compact form during the growth period of the spermatocyte, and so is not recognizable there; but in the first maturation mitosis it is immediately recognizable as the only chromosome that has not conjugated with another to form a bivalent one. Now such a chromosome of *Alydus*, *Harmostes* and *Edancala* agrees with the chromosome X of *Protenor* in not pairing with another during the synapsis stage; but differs from it in behaving like the other chromosomes after a mitosis, *i. e.*, in losing its compact structure and strong affinity for chromatin stains.

Why should a heterochromosome be sometimes unpaired in the

spermatogonia, sometimes paired? When they are unpaired they are larger than the other chromosomes. This might imply that such an unpaired heterochromosome really represents two in close union, *i. e.*, is already bivalent in the spermatogonium. And this I think is a very probable explanation, in view, first, of the behavior of the unpaired chromosomes during the growth period of the spermatocyte in *Protenor* (fully described in my paper, "The Germ Cells of the Metazoa"); and, second, of the fact that such a chromosome sometimes shows a distinct constriction at its middle (shown for *Harmostes* in my paper, "Further Studies on the Chromosomes," etc.).

It is hoped that these considerations, in endeavoring to show the likenesses and differences of the two kinds of heterochromosomes, will bring more uniformity in the interpretation of these modified chromosomes, and that such chromosomes should always be taken into account in any discussion of the idea of the individuality of the chromosomes.

4. THE CONJUGATION OF THE CHROMOSOMES IN THE SYNOPSIS STAGE, AND ITS RELATION TO THE REDUCTION DIVISION.

It is now determined for a considerable number of cases that in the early portion of the growth period of both ovocytes and spermatocytes there takes place the process known as the "reduction in number" of the chromosomes. Thus if there are twenty-four chromosomes in the spermatogonium, twelve are found in the maturation period before the first mitosis. This fact was first established by Boveri ("Zellen-Studien," *l. c.*) and by Brauer.¹ Really the name applied is a misnomer, for there is no loss of chromosomes, no true "reduction" in this number, but it is a conjugation of the chromosomes. Rückert² sought to explain it by stating that in the prophase of the first maturation division the chromatin spirem breaks into only half the normal number of segments. This, however, is inadequate as an explanation, for I showed in the "Spermatogenesis of Peripatus" that in the prophases of the first maturation mitosis there is a

¹"Zur Kenntniss der Spermatogenese von *Ascaris megalocephala*," *Arch. Mikr. Anat.*, 42, 1893.

²"Zur Eireifung bei Copepoden," *Anat. Hefte*, 4, 1894.

continuous *linin* spirem, which probably does not break into segments until the metakinesis of the first maturation mitosis, but no continuous *chromatin* spirem. Hence it is not a question of chromosomes which were already contiguous remaining contiguous, but of chromosomes which were first separated (except for their linin connections) conjugating to form pairs during the synapsis stage. The criterion of the synapsis stage is such a pairing; and the term "conjugation" of the chromosomes represents the facts much better than the term "reduction in number."

The bivalent chromosomes so formed by conjugation, in the Hemiptera, *Peripatus* and Amphibia, become so placed in the equator of the spindle of the first maturation mitosis, that entire univalent chromosomes become separated. This is a true reduction division in the sense of Weismann. Each spermatocyte of the second order thus receives whole univalent chromosomes in one half the normal (somatic) number. While the majority of writers still hold that no such reduction division occurs, the idea being abhorrent to them, there are still a number who have furnished an array of facts that can be interpreted only as speaking for such an occurrence; thus Rückert, Häcker, Vom Rath, Korschelt, Henking, Paulmier, McClung, Sutton, Nichols, Griffin, Van Winiwarter, Lillie, Schockaert. But the arraying of names on the one side against those on the other is no argument in itself, and we may pass to the discussion of certain facts which harmonize completely with the occurrence of a reduction division, and remain unexplainable on any other basis.

First, it may be recalled that there is a divergence of opinion as to which of the two maturation mitoses is the reduction division, some holding that it is the first and others, the second. There is no good reason, save the probability that there would be expected uniformity in such important processes, that this division should always be in the first mitosis, or always in the second; for it is quite possible that there is a difference in this regard in different objects. In the discussion which follows we will assume it to be the first maturation mitosis since there occurs the reduction division in the objects specially studied by me.

Now I reached the conclusion ("A Study of the Chromosomes," etc.) that in the synapsis stage there is effected a conjugation of paternal with maternal chromosomes; under "paternal" understanding those derived from the spermatozoön, and under "maternal," those from the ovotid. The arguments for this were stated as follows:

1. In *Ascaris megaloccephala univalens* there is the normal number of two chromosomes. The ovotid and spermatid have each only one. In the fertilized egg there is one derived from the spermatid, one from the ovotid; therefore the bivalent chromosome found in the maturation period of the spermatocyte or ovocyte must have been formed by the conjugation of a paternal with a maternal chromosome.

2. In the spermatogenesis of the Hemiptera there are usually two small heterochromosomes in the spermatogonia. These unite to form a bivalent one in the spermatocyte. They become separated from each other in the first maturation mitosis so that no spermatid receives more than one. Evidently then in the fertilized ovum since only one comes from the spermatid, the other must come from the ovitid. Therefore in the conjugation of the two in the synapsis, it is a conjugation of a paternal one with a maternal one. That was reasoned out without any knowledge of such chromosomes of the ovogenesis. Now I add Fig. 15, showing among the chromosomes of an ovogenic monaster stage the two small elements N and n , which are heterochromosomes of the same number and size as those found in the spermatogonium (Fig. 16, N , n). Therefore, there must be a conjugation in the ovogenetic synapsis stage, as well as in the spermatogenetic, of a paternal heterochromosome with a maternal one.

3. That besides the heterochromosomes, whenever there is recognizable in the spermatogenic chromosomal plate a pair of chromosomes notably different from the others in volume, there is always found in the first maturation mitosis a particular bivalent chromosome notably different in volume from the other ones, and so evidently formed in the synapsis by the conjugation of the two peculiar univalent ones of the spermatogonium. This bivalent chromosome is so placed in the first maturation spindle

that its two univalent elements pass to opposite cells, so the spermatid has never more than one of them. One of those in the spermatogonium must accordingly have come from the spermatid and one from the ovoid, which combined to give rise to that spermatogonium. And here, also, in the formation of such a bivalent chromosome in the synapsis there must be a union of a paternal with a maternal chromosome. No other explanation seems possible.

These conclusions, the numerical ratios of certain clearly distinguishable chromosomes in the spermatogonia, to certain equally distinguishable ones in the spermatocytes and spermatids, could be established for the heterochromosomes for some forty species of Hemiptera, and for other chromosomes in the cases of *Trichpepla semivittata*, *Protenor belfragei*, *Peliopelta abbreviata*, *Prionidus cristatus*, *Zaitha fluminea* and *Corizus lateralis*. To make this point clear a few figures of certain of these cases are given here again. In the spermatogonium of *Anasa* (Fig. 16), as well as in the ovogonium (Fig. 15), are recognizable among the 22 chromosomes, two very much smaller than the others (N, n , heterochromosomes), and two considerably larger (A, a); in the first spermatocyte there are eleven bivalent ones, six of which are shown in Fig. 17, and here we recognize again the chromosomes N, n and A, a . In *Corizus*, in the spermatogonium (Fig. 18) are two particularly small (N, n) and two particularly large chromosomes; and these recognizable again in the first maturation spindle (Fig. 19). Similarly in the case of *Trichpepla* (Figs. 20, 21). In the spermatogonium of *Protenor* (Fig. 22) are thirteen chromosomes; a particularly large one (X), two next in size (K, k), and two smallest (N, n). In the spermatocyte (Fig. 23) X is recognizable as being the largest; it is the odd chromosome and is not paired with any other. N and n are paired and so are K and k .

From these observations I concluded that probably in every case the chromosomes in the synapsis united to form bivalent pairs in such a way that the one of each pair was paternal and the other maternal; and I was able actually to demonstrate it in those cases where the differences in volume between the chromosomes were sufficient to allow them to be followed from genera-

tion to generation. And I could also prove that in all cases the two components of each bivalent chromosome always become separated from each other in the first maturation mitosis.

Following this came the paper of Sutton (*l. c.*), proving conclusively that in the spermatogonium of *Brachystola* the chromosomes occur regularly in pairs of graduated sizes, the two of a pair being always of the same length; that in the synapsis stage bivalent chromosomes are produced by the conjugation of every two chromosomes of the same length; and that corresponding chromosomes became separated from each other in the reduction division (here the second maturation mitosis). So he concluded quite rightly that there are two series of chromosomes in the spermatogonium, a paternal series, $A, B, C \dots n$, and a maternal series, $a, b, c \dots n$, in which A corresponds to a in size and hereditary value, B to b , and so through the series. By A joining with a in the synapsis, like chromosomes conjugate; and by these separating from each other in the reduction divisions, it results that two chromosomes of like size are not found in the spermatid. Sutton was the first to demonstrate this for the whole series of chromosomes, and to argue that such a conjugation, together with the following reduction division, would operate so that no spermatid could receive two chromosomes of like hereditary value, but only one chromosome representing a particular value.

In the present paper I have shown that in *Plethodon* and *Desmognathus* also one may recognize the two corresponding series of chromosomes in the spermatogonium. An examination of *Ascaris megalocephala bivalens* shows the same relation. Pole views of the first cleavage spindle (Figs. 28-30) show each two larger (A, a) and two smaller chromosomes (B, b). The differences in size of the two pairs is not very great, but always recognizable when the chromosomes can be seen in their entirety. This is then evidently a case parallel to those described above: that of the larger pair (A, a) one is paternal and the other maternal, and that of the smaller pair (B, b) the same relation holds. Now the formation of the tetrads in the ovogenesis of this species has been described by Boveri as two equational (longitudinal) divisions of each bivalent chromosome; and Braur has reached the same result for the formation of the tetrads in the spermatogen-

esis. But in concluding this these writers do not give a satisfactory explanation either why or how univalent chromosomes unite to form bivalent ones, and so give no clue to a reason for the chromosomes being paired in the fertilized egg. This point can be settled only by a careful reëxamination of the changes in the early growth period, and here I shall simply call attention to certain appearances that speak for the first maturation mitosis in *Ascaris* being a reduction division.

In the fertilized egg are two pairs of chromosomes, *A*, *a* and *B*, *b* (Figs. 28–30), one pair being considerably larger than the other, and the two composing a pair sometimes differing somewhat in length but being approximately equal in volume. As Van Beneden first showed, two of these chromosomes come from the ovotid, and two from the spermatozoön. Fig. 27 shows a slightly earlier stage, the chromosomes in two groups, one group derived from the male pronucleus and the other from the female pronucleus. In the group to the right is a large and a small chromosome (*A*, *B*); in the group to the left also a large and small one (*a*, *b*). But *A* corresponds approximately to *a* in volume, and *B* to *b*. Therefore we may say that of the four chromosomes in the fertilized egg (Figs. 27–30) a small paternal one (from the male pronucleus) corresponds in volume to a small maternal one (from the female pronucleus), and a large paternal one to a large maternal one. In other words, of each pair of chromosomes in the fertilized egg, one chromosome is paternal and one maternal. Which two come from the male pronucleus, and which two from the female pronucleus, there is as yet no means of deciding, for the two pronuclei appear structurally alike. But to make my argument clear I will assume that *A* and *B* are paternal, and *a* and *b* maternal.

Now in the formation of the first polar body (Figs. 24, 25) we find the two well-known quadripartite chromosomes. There are two, not four; hence they are bivalent with regard to the normal number. In each bivalent chromosome (tetrad) we should expect then to find two univalent chromosomes. Both these figures (24, 25) were drawn with great care to get the exact proportions of the parts of the chromosomes; both represent the stage where one plate of chromosomes is passing into the polar body.

Now in each of these cases we notice in the polar body, as in the egg, a larger and a smaller bipartite chromosome. Thus in the polar body the larger dyad A, A , and the smaller B, B ; in the egg the larger dyad a, a , and the smaller, b, b . As far as I can determine this relation appears to be constant: one large and one small dyad in the polar body as well as in the egg; and not two smaller (or larger) dyads in the polar body and two larger (or smaller) dyads in the egg. Now from what we have found to be the case in other objects, I would judge A, A to be an entire univalent chromosome that had been paired previously with the entire univalent chromosome a, a ; and that a similar relation holds between B, B and b, b . This would then be a reduction division, separating entire univalent chromosomes. In favor of this is the fact that A, A is approximately similar in volume to a, a , and B, B to b, b ; and we have learned that chromosomes of similar volumes conjugate in synapsis. Two dyads are left in the egg, a, a and b, b , each of which could be regarded as a longitudinally split univalent chromosome. In the formation of the second pole body (Fig. 26) the two parts of each dyad separate from each other, and this would be an equational division. There are then left in the egg the two chromosomes a and b , which differ markedly in volume. And this is in exact accord with the fact shown in Fig. 27, that from each pronucleus comes one large and one small chromosome.

This interpretation would bring *Ascaris* into close agreement with the other objects discussed in this paper: it explains why there are two large and two small chromosomes in the fertilized egg; why each pronucleus has one large and one small chromosome; finally, why the two bivalent chromosomes of the first maturation mitosis differ in volume. The idea that each such bivalent chromosome has been formed by a double longitudinal splitting, hence that both divisions are equational, gives no explanation for any of these phenomena, nor yet explains how or why bivalent chromosomes should be formed. The onus no longer rests upon us to prove the occurrence of a reduction division; but upon those of the other school to prove that a bivalent chromosome represents one chromosome that has undergone a double longitudinal division, and to show that such an interpre-

tation is explanatory of the kind of phenomena that we have discussed.

5. CHROMOSOMAL COMBINATIONS AND THE MENDELIAN RATIO.

In his paper on "The Chromosomes in Heredity"¹ Sutton argues that the combination of paternal and maternal chromosomes in the fertilized egg would result in a Mendelian ratio. It will be recalled that Mendel² in his experiments on crossing varieties of *Pisum*, to determine the law of transmission of parental characters to the hybrid, found the crosses to result in the ratio 1 *D* : 2 *Dr* : 1 *r*, in which *D* represents the pure character of one parent, *r* the pure character of the other parent, and *Dr* represents the possession of both characters. In other words: out of four offspring resulting from such a cross, one would resemble the father, one the mother, and two combine the characters of both parents.

Sutton starts with the fact that there are two series of chromosomes in each fertilized egg, *A, B, C, ... n* and *a, b, c, ... n*, the first set of paternal origin (from the spermatozoön) and the second of maternal (from the ovotid). In these series *A* is the homologue of *a*, *B* of *b*, and so on. In the synapsis stage of the germ cells *A* would conjugate with *a*, *B* with *b*, and so on, so there would be formed the bivalent chromosomes *Aa, Bb, Cc, ... n*. In the reduction division *A* and *a* would pass to separate cells, and such would be the case with *B* and *b* and the remaining paired chromosomes.

Then Sutton takes the case where there are the two homologous chromosomes *A* and *a* in an ovogonium and *A* and *a* in a spermatogonium; in the maturation period there would be formed *Aa* in the ovocyte, and *Aa* in the spermatocyte; the ovotids would contain then either *A* or *a*, and the spermatids either *A* or *a*. There would then result in fertilization these combinations:

$$\begin{aligned} A\delta + A\varphi &= AA \\ A\delta + a\varphi &= Aa \\ a\delta + A\varphi &= aA \\ a\delta + a\varphi &= aa. \end{aligned}$$

¹ BIOLOG. BULL., 4, 1903.

² "Versuche über Pflanzenhybriden," *Verh. nat. Ver. Brünn*, 4, 1865.

"Since the second and third of these are alike the result would be expressed by the formula $AA:2Aa:aa$ which is the same as that given for any character in a Mendelian case."

But as a matter of fact this can be so only in a case where there are only two chromosomes in the fertilized egg. For let us take the case where the normal number of chromosomes is four; express by capital letters those chromosomes originally derived from the spermatozoon, and by small letters those derived from the ovid; and assume that A is homologous to a , and B to b . Then the spermatogonium would have the chromosomes, A, a, B, b , and the ovogonium have also A, a, B, b . By the synapsis stage would be formed bivalent chromosomes Aa, Bb in the spermatocyte, and Aa, Bb in the ovocyte. The reduction division would separate A from a and B from b in both spermatogenesis and oogenesis. The spermatids would contain then either A, B , or a, b , or A, b , or a, B ; and the ootids either A, B or a, b , or A, b , or a, B . In the fertilization of one of these ootids by one of the spermatozoa, 16 different combinations are possible: A, B, A, B ; A, B, a, b ; A, B, A, b ; A, B, a, B ; a, b, A, B ; a, b, a, b ; a, b, A, b ; a, b, a, B ; A, b, A, B ; A, b, a, b ; A, b, A, b ; A, b, a, B ; a, B, A, B ; a, B, a, b ; a, B, A, b ; a, B, a, B . But only one of these combinations is of purely paternal chromosomes, namely A, B, A, B ; and only one of purely maternal, namely a, b, a, b . The other fourteen combinations show paternal together with maternal chromosomes (six cases where the paternal and maternal chromosomes are present in equal number, four cases where there are three paternal chromosomes to one maternal, and four cases where there are three maternal chromosomes to one paternal).

Hence the ratio is $1P:14PM:1M$, where P stands for purely paternal chromosomes, M for purely maternal, and PM for combinations of paternal and maternal chromosomes. This is clearly not a Mendelian ratio of $1:2:1$. And obviously the disparity would become greater with any increase in the number of chromosomes. According to Sutton's own computation, in forms which have 24 chromosomes, the number of possible combinations of these in the fertilized egg would be 16,777,216. That would give the ratio of $1P:16,777,214PM:1M$.

But though the combinations of paternal and maternal chromosomes in the fertilized egg do not support the Mendelian ratio for hybrids, I fully agree with Sutton that "the phenomena of germ-cell divisions and of heredity are seen to have the essential features, viz., purity of units (chromosomes, characters) and the independent transmission of the same."

UNIVERSITY OF TEXAS,
November 29, 1903.

EXPLANATION OF FIGURES.

FIGS. 1-3. Pole views of spermatogonic monasters of *Plethodon cinereus*.

FIG. 4. Pole view of a spermatogonic monaster of *Diemyctilus virescens*.

FIGS. 5, 6. Pole and lateral views respectively of spermatogonic monasters of *Desmognathus fuscus*.

FIG. 7. Oblique pole view of one plate of daughter chromosomes, early spermatogonic anaphase of *Desmognathus fuscus*.

FIG. 8. Lateral view of a late synapsis (postsynapsis) stage of *Desmognathus fuscus*; four entire bivalent chromosomes shown, and half of another. Nuclear membrane not yet formed.

FIG. 9. Polar view of a spermatocytic nucleus at a slightly later stage in the same species.

FIGS. 10-12. Successive prophases of the first maturation mitosis in *Desmognathus fuscus*.

FIG. 13. Lateral view of a spindle of the first maturation mitosis, showing three bivalent chromosomes in metakinesis; *Plethodon cinereus*.

FIG. 14. A heterotypic chromosome of *Plethodon cinereus* in its definitive form.

FIG. 15. *Anasa* (undetermined species from California), pole view of ovogonic monaster.

FIG. 16. *Anasa* sp., pole view of spermatogonic monaster.

FIG. 17. *Anasa* sp., lateral view of first maturation mitosis, showing six of the eleven bivalent chromosomes.

FIG. 18. *Corizus alternatus*, pole view of the spermatogonic monaster.

FIG. 19. *Corizus alternatus*, lateral view of first maturation mitosis, showing five of the bivalent chromosomes.

FIG. 20. *Trichpepla semivittata*, pole view of spermatogonic monaster.

FIG. 21. *Trichpepla semivittata*, lateral view of first maturation mitosis, showing all the bivalent chromosomes.

FIG. 22. *Protenor belfragei*, pole view of spermatogonic monaster.

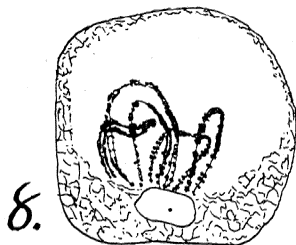
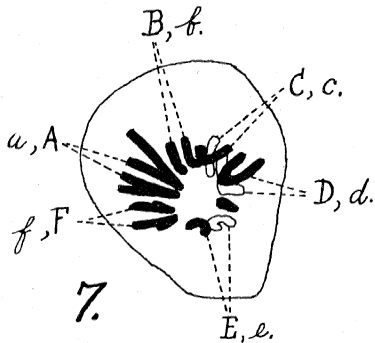
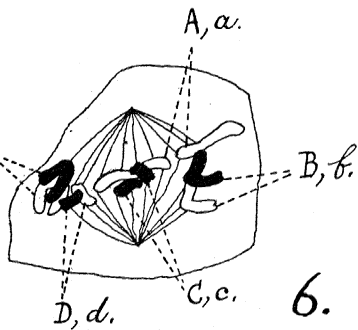
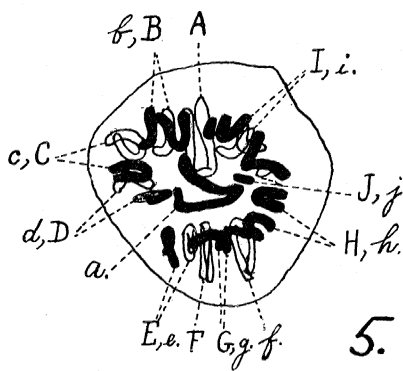
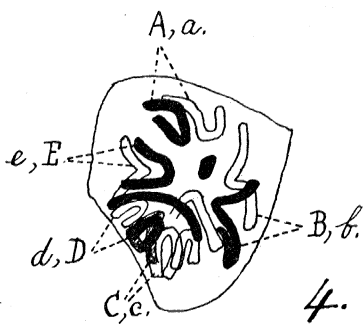
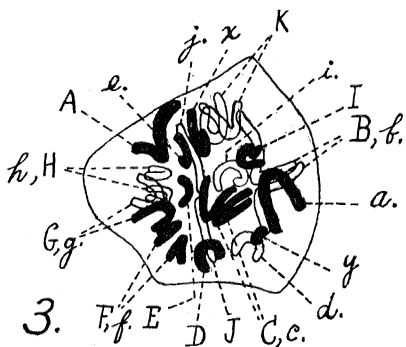
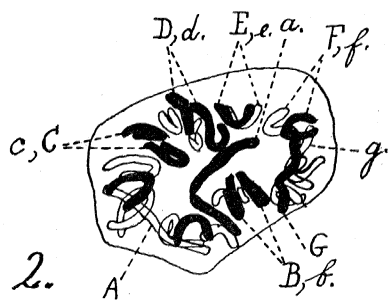
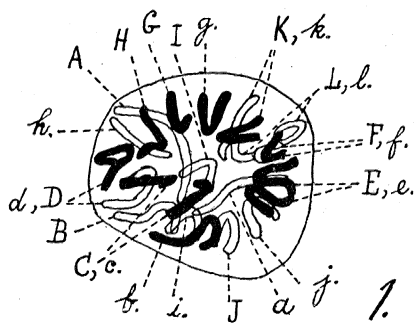
FIG. 23. *Protenor belfragei*, late prophase of the first maturation mitosis.

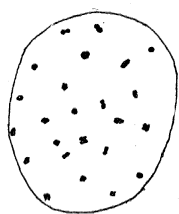
FIGS. 24, 25. *Ascaris megalocephala bivalens*, formation of the first polar body (first maturation mitosis), the spindle seen very obliquely in Fig. 25.

FIG. 26. *Idem*, formation of second polar body.

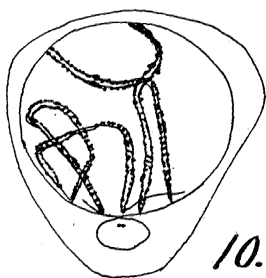
FIG. 27. *Idem*, lateral view of the first cleavage spindle showing the two groups of chromosomes.

FIG. 28-30. *Idem*, pole views of the chromosomes in the first cleavage spindle.

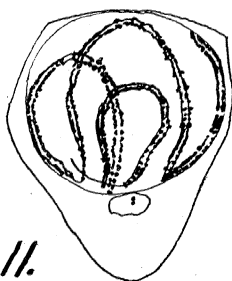




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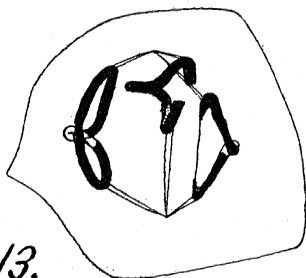
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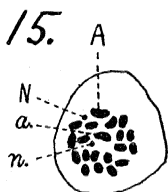
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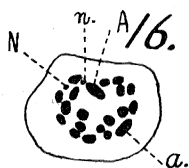
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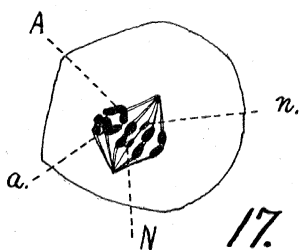
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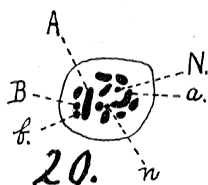
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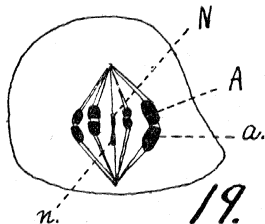
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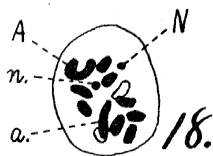
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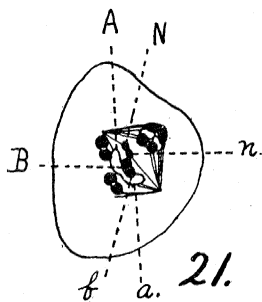
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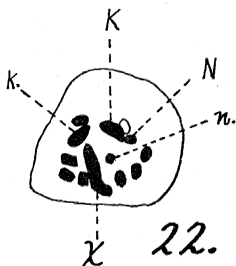
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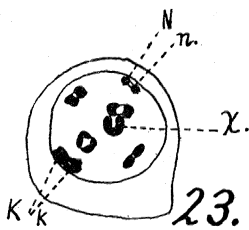
18.



21.



22.



23.

